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COMPOSITIONS AND METHODS FOR THE TREATMENT OF NATURAL KILLER CELL RELATED DISEASES

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Field of the Invention

The present invention relates to compositions and methods useful for the diagnosis and treatment of immune related diseases.

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Background of the Invention

Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

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Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

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Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, *etc.*

Immune related diseases could be treated by suppressing the immune response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

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Natural killer (NK) cells are an important effector cell of the innate immune system. They are specialized to effect killing against host cells that have either been infected by viruses, parasites or that have become cancerous. Phenotypically, NK cells are large granular lymphocytes that constitute ~2 % of the circulating lymphocyte population. They are commonly identified by cell surface expression of CD56 and CD16. NK cells mature in the bone marrow from a CD34+ precursor cell that they share with T cells. The mature NK cell, shares expression of CD8, cytolytic machinery, and some KIRs, with T cells, but remains distinct from T cells by the lack of CD3 and the T cell receptors. Like cytotoxic T cells, they contain granules filled with pore forming protein, cytotoxins, serine esterases and proteoglycans that mediate lysis of target cells. Both cytotoxic T cells and NK cells kill on contact by binding to their targets and delivering their lethal burst of chemicals that produces holes in the target cell's membrane. Unlike cytotoxic T cells, NK cells do not need to recognize a specific antigen before initiating lysis. Rather, NK cell activation can be mediated by growth factors and cytokines such as, IL-2, IL-12 and IL-15 have been shown to mediate

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proliferative and cytotoxic activities or by a delicate balance between two classes of NK cell receptors, one that activates the cells, and another that inhibits. Killer Ig-like receptors (KIRs) are NK cell receptors that transmit an inhibitory signal if they encounter class I MHC molecules on a cell surface. This is important for killing of both cancerous cells and virally infected cells. Because viruses often suppress class I MHC expression in cells they infect, the virus-infected cell becomes susceptible to killing by NK cells. Likewise, cancer cells have reduced or no class I MHC expression also become susceptible to killing by NK cells. Natural cytotoxicity receptors (NCRs) constitute a family of activating receptors on NK cells. In some effector-target systems, the surface density of NCRs correlates with the cytolytic activity of the NK cells, while in other systems killing requires cooperation between NCR, another activating receptor NKG2D and its adaptor polypeptide DAP10. Additionally, the strength of the stimulatory signals can be influenced by engagement of co-receptors such as 2B4 and NTB-A. The ligands for NCRs and NKG2D, hemoglutanins and MICA, MICB respectively are not expressed by most normal cells, but are induced in most tumor cell lines. Expression of the ligands by tumor cells triggers a dramatic immune response resulting in tumor cell rejection.

Activation of NK cells with IL-15 or IL-12 have been shown to induce both cytotoxic and proliferative effects. Junctional adhesion molecule 2 (JAM2) has been shown to bind to NK cells and has been hypothesized to play a role in lymphocyte extravasation to sites of inflammation. Therefore, a DNA microarray experiment comparing differential expression of genes from these three modes of activation versus resting NK cells has the potential to reveal novel genes or novel gene associations with NK cell activity. Therapeutic antibodies, peptides or small molecules could be developed to target specific genes revealed by these microarrays for the treatment of immune mediated inflammatory diseases and malignancies.

Despite the above research in NK cells, there is a great need for additional diagnostic and therapeutic agents capable of detecting the presence of NK cell mediated disorders in a mammal and for effectively reducing these disorders. Accordingly, it is an objective of the present invention to identify polypeptides that are differentially expressed in activated NK cells as compared to resting NK cells, and to use those polypeptides, and their encoding nucleic acids, to produce compositions of matter useful in the therapeutic treatment and diagnostic detection of NK cell mediated disorders in mammals.

Summary of the Invention

A. Embodiments

The present invention concerns compositions and methods useful for the diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which are a result of stimulation of the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the immune response to an antigen (*e.g.*, neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (*e.g.*, inflammation).

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Accordingly, the PRO polypeptides, agonists and antagonists thereof are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. In a specific aspect, such medicines and medicaments comprise a therapeutically effective amount of a PRO polypeptide, agonist or antagonist thereof with a pharmaceutically acceptable carrier. Preferably, the admixture is sterile.

5 In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide. In a specific aspect, the PRO agonist or antagonist is an anti-PRO antibody.

10 In another embodiment, the invention concerns a composition of matter comprising a PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition comprises a therapeutically effective amount of the polypeptide or antibody. In another aspect, when the composition comprises an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, (c) increasing the proliferation of NK cells in a mammal in need thereof in response to an antigen, (d) stimulating the activity of NK cells or (e) increasing the vascular permeability. In a further aspect, when the composition comprises an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, (c) decreasing the activity of NK cells or (d) decreasing the proliferation of NK cells in a mammal in need thereof in response to an antigen. In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

25 In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO polypeptide, an agonist thereof, or an antagonist thereto. In a preferred aspect, the immune related disorder is selected from the group consisting of: systemic lupus erythematosus, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease.

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In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic antibody.

In yet another embodiment, the present invention provides a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Alternatively, the antibody is a monoclonal antibody, an antibody fragment, a humanized antibody, or a single-chain antibody.

In a further embodiment, the invention concerns an article of manufacture, comprising:

- (a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;
- (b) a container containing said composition; and
- (c) a label affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

In yet another embodiment, the present invention concerns a method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and a PRO polypeptide, in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

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In another embodiment, the invention provides a method for determining the presence of a PRO polypeptide in a sample comprising exposing a test sample of cells suspected of containing the PRO polypeptide to an anti-PRO antibody and determining the binding of said antibody to said cell sample. In a specific aspect, the sample comprises a cell suspected of containing the PRO polypeptide and the antibody binds to the cell. The antibody is preferably detectably labeled and/or bound to a solid support.

In another embodiment, the present invention concerns an immune-related disease diagnostic kit, comprising an anti-PRO antibody and a carrier in suitable packaging. The kit preferably contains instructions for using the antibody to detect the presence of the PRO polypeptide. Preferably the carrier is pharmaceutically acceptable.

In another embodiment, the present invention concerns a diagnostic kit, containing an anti-PRO antibody in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO polypeptide.

In another embodiment, the invention provides a method of diagnosing an immune-related disease in a mammal which comprises detecting the presence or absence of a PRO polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of the PRO polypeptide in said test sample is indicative of the presence of an immune-related disease in said mammal.

In another embodiment, the present invention concerns a method for identifying an agonist of a PRO polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, wherein the induction of said cellular response is indicative of said test compound being an effective agonist.

In another embodiment, the invention concerns a method for identifying a compound capable of inhibiting the activity of a PRO polypeptide comprising contacting a candidate compound with a PRO polypeptide under conditions and for a time sufficient to allow these two components to interact and determining whether the activity of the PRO polypeptide is inhibited. In a specific aspect, either the candidate compound or the PRO polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

In another embodiment, the invention provides a method for identifying a compound that inhibits the expression of a PRO polypeptide in cells that normally express the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO polypeptide is inhibited. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO polypeptide; and

(b) determining the inhibition of expression of said polypeptide.

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In yet another embodiment, the present invention concerns a method for treating an immune-related disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide or (c) an antagonist of a PRO polypeptide, wherein said agonist or antagonist may be an anti-PRO antibody. In a preferred embodiment, the mammal is human. In another preferred embodiment, the nucleic acid is administered via *ex vivo* gene therapy. In a further preferred embodiment, the nucleic acid is comprised within a vector, more preferably an adenoviral, adeno-associated viral, lentiviral or retroviral vector.

In yet another aspect, the invention provides a recombinant viral particle comprising a viral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the viral vector is in association with viral structural proteins. Preferably, the signal sequence is from a mammal, such as from a native PRO polypeptide.

In a still further embodiment, the invention concerns an *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

In a still further embodiment, the invention provides a method of increasing the activity of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of NK cells in the mammal is increased.

In a still further embodiment, the invention provides a method of decreasing the activity of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of NK cells in the mammal is decreased.

In a still further embodiment, the invention provides a method of increasing the proliferation of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of NK cells in the mammal is increased.

In a still further embodiment, the invention provides a method of decreasing the proliferation of NK cells in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of NK cells in the mammal is decreased.

B. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions

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suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid

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sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160

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nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400

5 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length.

10 It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments

15 encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences herein above identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid

20 sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about

25 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid

30 sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length

35 amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity,

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sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as herein before described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as herein before described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

In the list of figures for the present application, specific cDNA sequences which are differentially expressed in activated Natural Killer (NK) cells as compared to normal resting NK cells are individually identified with a specific alphanumeric designation. These cDNA sequences are differentially expressed in NK cells that are specifically treated as described in Example 1 below. If start and/or stop

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codons have been identified in a cDNA sequence shown in the attached figures, they are shown in bold and underlined font, and the encoded polypeptide is shown in the next consecutive figure.

The Figures 1-1477 show the nucleic acids of the invention and their encoded PRO polypeptides. Also included, for convenience is a List of Figures attached hereto as Appendix A, which gives the figure number and the corresponding DNA or PRO number.

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 Figure 74: PRO82678
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 Figure 76: PRO84752
 Figure 77: DNA329104, NP_004085.1, 201144.s_at
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 Figure 85: DNA103488, NP_002583.1, 201202.at
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 Figure 99: DNA331290, NP_038474.1, 201285.at
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Figure 603: DNA329552, NP_063948.1, 208925_at
Figure 604: PRO85097
Figure 605: DNA328629, NP_006079.1, 208977_x.at
Figure 606: PRO84407
Figure 607: DNA330154, HUMPECAM27, 208981_at
Figure 608: DNA330155, 7692317.2, 208982_at
Figure 609: PRO85407
Figure 610: DNA328631, AK027318, 209006_s.at
Figure 611: PRO84409
Figure 612: DNA328632, DJ465N24.2.1Homo, 209007_s.at
Figure 613: DNA328635, BC020946, 209026_x.at
Figure 614: PRO84413
Figure 615: DNA274202, NP_006804.1, 209034_at
Figure 616: PRO62131
Figure 617A-C: DNA328637, HSA7042, 209053_s.at
Figure 618: PRO81109
Figure 619: DNA327713, BC010653, 209146_at
Figure 620: PRO37975
Figure 621A-B: DNA328642, AF073310, 209184_s.at
Figure 622: PRO84418
Figure 623: DNA331331, AF161416, 209185_s.at
Figure 624A-B: DNA328643, HUMHK1A, 209186_at
Figure 625: PRO84419
Figure 626: DNA103520, NP_002639.1, 209193_at
Figure 627: PRO4847
Figure 628: DNA226176, NP_003458.1, 209201_x.at
Figure 629: PRO36639
Figure 630: DNA326267, NP_004861.1, 209208_at
Figure 631: PRO82674
Figure 632: DNA328645, NP_009006.1, 209216_at
Figure 633: PRO84421
Figure 634: DNA227483, NP_003120.1, 209218_at
Figure 635: PRO37946
Figure 636: DNA331332, BC007405, 209238_at

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 Figure 638A-B: DNA331333, 371440.32, 209240.at
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 Figure 640: DNA328649, NP_116093.1, 209251.x.at
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 Figure 642: DNA255255, NP_071437.1, 209267.s.at
 Figure 643: PRO50332
 Figure 644: DNA269750, NP_002919.1, 209324.s.at
 Figure 645: PRO58159
 Figure 646: DNA269750, RGS16, 209325.s.at
 Figure 647: PRO58159
 Figure 648: DNA269630, NP_003281.1, 209344.at
 Figure 649: PRO58042
 Figure 650: DNA330170, AF109161, 209357.at
 Figure 651: PRO84807
 Figure 652: DNA253811, NP_004410.2, 209457.at
 Figure 653: PRO49214
 Figure 654: DNA324899, NP_002938.1, 209507.at
 Figure 655: PRO81503
 Figure 656: DNA328663, NP_057157.1, 209524.at
 Figure 657: PRO36183
 Figure 658: DNA328663, CGI-142, 209526.s.at
 Figure 659: PRO36183
 Figure 660A-B: DNA328670, BC001618, 209610.s.at
 Figure 661: PRO70011
 Figure 662: DNA328599, HSNFKBS, 209636.at
 Figure 663: PRO84382
 Figure 664: DNA330186, NP_004327.1, 209642.at
 Figure 665: PRO85434
 Figure 666: DNA330191, NP_036249.1, 209715.at
 Figure 667: PRO85439
 Figure 668: DNA329178, HSU90552, 209770.at
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 Figure 670: DNA329205, NP_001343.1, 209782.s.at
 Figure 671: PRO84821
 Figure 672: DNA226436, NP_001772.1, 209795.at
 Figure 673: PRO36899
 Figure 674A-B: DNA196499, AB002384, 209829.at
 Figure 675: PRO24988
 Figure 676: DNA330197, NP_112190.1, 209832.s.at
 Figure 677: PRO85445
 Figure 678: DNA331334, AF117233, 209845.at
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 Figure 681: PRO61867
 Figure 682: DNA330198, AB014719, 209871.s.at
 Figure 683: PRO85446
 Figure 684: DNA154921, DNA154921, 209967.s.at
 Figure 685A-B: DNA227134, NP_000918.1, 209993.at
 Figure 686: PRO37597
 Figure 687: DNA226658, NP_003736.1, 209999.x.at
 Figure 688: PRO37121
 Figure 689: DNA226658, SSI-1, 210001.s.at
 Figure 690: PRO37121
 Figure 691A-B: DNA328685, NP_127497.1, 210113.s.at
 Figure 692: PRO34751
 Figure 693: DNA330203, NP_003755.1, 210190.at
 Figure 694: PRO85449
 Figure 695: DNA331335, AF070576, 210202.s.at
 Figure 696: DNA217253, NP_000749.1, 210229.s.at
 Figure 697: PRO34295
 Figure 698: DNA328690, NP_524145.1, 210240.s.at
 Figure 699: PRO59660
 Figure 700: DNA330206, NP_005801.2, 210288.at
 Figure 701: PRO85450
 Figure 702: DNA225528, NP_000610.1, 210354.at
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 Figure 704: DNA331336, TANK, 210458.s.at
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 Figure 706: DNA329217, BC003406, 210571.s.at
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 Figure 708: DNA330210, HSU03858, 210607.at
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 Figure 710: DNA331337, TNFSF11, 210643.at
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 Figure 712: DNA227446, NP_003551.1, 210647.x.at
 Figure 713: PRO37909
 Figure 714: DNA256521, NP_038459.1, 210690.at
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 Figure 716: DNA331338, AF188298, 210757.x.at
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 Figure 718: DNA237817, NP_001307.1, 210766.s.at
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 Figure 720A-B: DNA330216, NP_006445.1, 210778.s.at
 Figure 721: PRO85457
 Figure 722: DNA188234, NP_000630.1, 210865.at
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 Figure 724: DNA228132, LCE, 210868.s.at
 Figure 725: PRO38595
 Figure 726: DNA238565, MCM7, 210983.s.at
 Figure 727: PRO39210
 Figure 728: DNA326239, NP_006752.1, 210996.s.at
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 Figure 730: DNA325039, NP_004902.1, 211048.s.at
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 Figure 732: DNA288254, NP_006000.2, 211058.x.at
 Figure 733: PRO69536
 Figure 734: DNA288254, TUBA3, 211072.x.at
 Figure 735: PRO69536
 Figure 736: DNA188234, TNFSF6, 211333.s.at
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 Figure 738: DNA331339, B3GALT3, 211379.x.at
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 Figure 742: DNA331340, HUMCD26A, 211478.s.at
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 Figure 744: DNA226578, CCNG2, 211559.s.at
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 Figure 748: DNA327760, NP_114430.1, 211685.s.at
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 Figure 751: PRO10347
 Figure 752: DNA329225, EVI2B, 211742.s.at
 Figure 753: PRO84833
 Figure 754: DNA328649, TUBA6, 211750.x.at
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 Figure 756: DNA254725, NP_002257.1, 211762.s.at
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 Figure 758: DNA330226, AF198052, 211794.at
 Figure 759: PRO85463
 Figure 760: DNA227173, FYB, 211795.s.at
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 Figure 762A-B: DNA331342, DEFCAP, 211822.s.at
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 Figure 764: DNA331343, AK026398, 211824.x.at
 Figure 765: PRO86423
 Figure 766: DNA226881, FLI1, 211825.s.at
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 Figure 768: DNA226176, CXCR4, 211919.s.at
 Figure 769: PRO36639
 Figure 770: DNA272286, CAT, 211922.s.at
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 Figure 779: PRO69690
 Figure 780: DNA330236, 228447.20, 212071.s.at
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 Figure 790: DNA329182, BC016852, 212259.s.at
 Figure 791: PRO84805
 Figure 792: DNA150980, HUMMAC30X, 212279.at
 Figure 793: DNA150980, DNA150980, 212281.s.at
 Figure 794: PRO12566
 Figure 795: DNA253017, DNA253017, 212282.at
 Figure 796: PRO48926
 Figure 797: DNA328719, BC012895, 212295.s.at
 Figure 798: PRO84475
 Figure 799: DNA271103, NP_005796.1, 212296.at
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 Figure 805: DNA331346, BC011685, 212330.at
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 Figure 834: DNA151487, DNA151487, 212594.at
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 Figure 838: DNA328744, AF318364, 212680.x.at
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Figure 857: DNA330277, CAB45152.1, 213142.x.at
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Figure 869: DNA327795, BC014226, 213457.at
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Figure 877: DNA331357, BC010494, 213646.x.at
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Figure 903: DNA331360, AK022497, 214177.s.at
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Figure 924: PRO85528
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 Figure 1106: DNA331381, BA108L7.2, 220974_x.at
 Figure 1107: PRO86451
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 Figure 1112: DNA331382, CISH, 221223_x.at
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 Figure 1115: PRO85652
 Figure 1116: DNA328948, NP_110437.1, 221253_s.at
 Figure 1117: PRO84659
 Figure 1118: DNA326507, NP_112490.2, 221267_s.at
 Figure 1119: PRO82879
 Figure 1120: DNA329471, NP_110387.1, 221417_x.at
 Figure 1121: PRO85036
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 Figure 1123: PRO84902
 Figure 1124: DNA227303, NP_004322.1, 221479_s.at
 Figure 1125: PRO37766
 Figure 1126: DNA326221, NP_057179.1, 221521_s.at
 Figure 1127: PRO82634
 Figure 1128: DNA330457, NP_076944.1, 221559_s.at
 Figure 1129: PRO85658
 Figure 1130: DNA329318, IRO033793, 221564_at
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 Figure 1132: PRO1607
 Figure 1133: DNA329319, NP_005440.1, 221602_s.at
 Figure 1134: PRO1607
 Figure 1135: DNA330459, NP_060083.1, 221677_s.at
 Figure 1136: PRO50083
 Figure 1137: DNA328961, NP_443112.1, 221756_at
 Figure 1138: PRO84667
 Figure 1139: DNA328961, MGC17330, 221757_at
 Figure 1140: PRO84667
 Figure 1141: DNA331383, BC007588, 221769_at
 Figure 1142: DNA331384, AK026326, 221985_at
 Figure 1143: PRO86454
 Figure 1144: DNA330467, NP_060114.1, 221986_s.at
 Figure 1145: PRO85665
 Figure 1146: DNA254739, NP_068766.1, 221987_s.at
 Figure 1147: PRO49837
 Figure 1148: DNA257797, DNA257797, 222036_s.at
 Figure 1149: DNA257798, DNA257798, 222037_at
 Figure 1150: DNA325648, NP_037409.2, 222077_s.at
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 Figure 1154A-B: DNA331387, NP_008919.2, 222162_s.at
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 Figure 1156: DNA328977, NP_071344.1, 222216_s.at
 Figure 1157: PRO84678
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 Figure 1159: PRO82287
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 Figure 1163: PRO4984
 Figure 1164: DNA327942, NP_060596.1, 222642_s.at
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 Figure 1166: DNA327943, NP_055399.1, 222646_s.at
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 Figure 1198: PRO49387
 Figure 1199: DNA254416, NP_060915.1, 223209.s.at
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 Figure 1203A-B: DNA257461, MAIL, 223218.s.at
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 Figure 1223: DNA327200, NP_114156.1, 223836.at
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 Figure 1227: DNA331393, D83532, 223961.s.at
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 Figure 1235: DNA331394, MGC11316, 224482.s.at
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 Figure 1254A-B: DNA287330, AB032991, 224801.at
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Figure 1295A-B: DNA331401, 336865.4, 225700.at
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Figure 1306: PRO69630
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Figure 1322A-B: DNA331407, 198233.1, 226352.at
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Figure 1324A-B: DNA331409, AB051464, 226370.at
Figure 1325A-B: DNA330675, 177663.2, 226372.at
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Figure 1339: PRO83953
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Figure 1353: DNA329444, BC017821, 227278.at
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